

we also ran a pilot study using a tissue clearing and 3D immunolabeling method combined with light sheet microscopy. RESULTS/ANTICIPATED RESULTS: We would expect to see higher cFos activation for brain areas in the reward pathway [including the Nucleus Accumbens (NAc), Ventral Tegmental Area (VTA), Prefrontal Cortex (PFC)] in heroin animals compared to saline animals. We can also expect higher activation in more novel areas like the lateral hypothalamus. DISCUSSION/SIGNIFICANCE OF FINDINGS: If we are able to track OUD effects through imaging in mice and rats, this can help us find better diagnostics, therapeutics, and procedures to treat the disorder. We can also eventually have a human brain atlas that outlines these affected areas as well in order to gain a better understanding on OUD particularly in the human population.

61892

Systemic TLR3-targeting Combinatorial Chemokine Modulation Sensitizes Murine Tumors to PD-1 Blockade

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ABSTRACT IMPACT: This work will lead to improved efficacy of immunotherapy directly impacting the survival of patients with hard to treat cancers. OBJECTIVES/GOALS: Immune checkpoint inhibitors (ICI) are most effective against 'hot' tumors highly infiltrated with cytotoxic T lymphocytes (CTLs) but have not worked well in poorly infiltrated 'cold' tumors. Thus, we are working to achieve a pretreatment regimen that will create a favorable immune profile allowing more effective ?PD-1 therapy. METHODS/STUDY POPULATION: BALB/c or C57BL/6 mice were inoculated with CRC murine cells CT26 or MC38, respectively. Mice were inoculated by two injection types: subcutaneous (SC), for systemic therapy, or intraperitoneal (IP), for local therapy. Tumor-bearing mice were given a two dose course of CKM consisting of IFN-? and rintatolimod via IP injection. Following CKM administration, mice were treated with three doses of ?PD-1 via IP injection. Mice were monitored for the kinetics of tumor growth and survival following treatment. The tumor microenvironment of treated mice was analyzed for production of chemokines, inflammatory cytokines and immune cell infiltration. RESULTS/ANTICIPATED RESULTS: CKM consisting of combination IFN-? and rintatolimod, but neither monotherapy alone, sensitized murine CRC tumors to subsequent ?PD-1 treatment. In both CT26 and MC38 tumor-bearing mice, tumor growth was hindered by CKM plus ?PD-1 treatment, independently on the route of treatment (local or systemic). Mice which experienced complete tumor regression were protected from re-challenge with a dose of tumor cells double that of the initial inoculation. Sensitizing tumors to ?PD-1 did not require intratumoral CKM administration and was observed with systemic application at distant sites. In accordance with these observations we expect that systemic CKM will induce strong increases of total and tumor-specific CTL counts in the tumor tissues as measured by both PCR and flow cytometry. DISCUSSION/SIGNIFICANCE OF FINDINGS: CKM sensitizing cold tumors to ?PD-1 indicates that intratumoral CTLs are an important factor dictating therapeutic effectiveness, independent of other factors such as tumor mutational load. The benefit of the sequential short-term CKM followed by routine ?PD-1 make this strategy feasible for rapid inclusion of into routine immunotherapy plans.

68477

Pancreatic cancer cell extracellular vesicles drive the unfolded protein response in recipient normal pancreatic cells

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ABSTRACT IMPACT: This study advances our understanding of potentially key drivers in the early formation of pancreatic cancer, a disease with few treatment options and poor patient outcomes. OBJECTIVES/GOALS: Patients diagnosed with pancreatic ductal adenocarcinoma (PDAC) have a 5-year survival rate of ~9%. A key driver of poor patient outcomes is late-stage diagnosis. A better understanding of PDAC onset is needed. This study was developed to understand how extracellular vesicles may be involved in the early formation of PDAC. METHODS/STUDY POPULATION: Extracellular vesicles (EVs) were isolated from several human PDAC and normal pancreatic cell lines, using ultracentrifugation with filtration or size exclusion chromatography. We next treated normal pancreatic cell lines with cancer cell EVs (cEVs). Next generation sequencing was used to measure global gene expression changes after treatment. Validations were performed using qPCR and luciferase activity assays. Multi-omics characterization of EVs was accomplished using mass spectrometry based proteomics, metabolomics and lipidomics analysis. RESULTS/ANTICIPATED RESULTS: We found that normal cells upregulated a variety of stress response pathways in response to cEVs. Lipid synthesis was also severely downregulated in these cells. We further validated activation of the unfolded protein response (UPR) in normal cells treated with cEVs. Multi-omics characterization of cEVs identified several enriched proteins, lipids and metabolites which may play a role in the activation of the UPR. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our results indicate that cEVs induce stress, and in particular the UPR, in normal pancreatic cells. Long-term UPR can impact a variety of cancer hallmarks. The UPR can mediate progression of pancreatic intraepithelial neoplasia (PanIN) to PDAC. Our results highlight a potential role for cEVs to alter the function of normal cells, aiding disease onset.

68722

Role of ER calcium in beta cell senescence and diabetes pathophysiology

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ABSTRACT IMPACT: The proposed study has the potential to inform new paradigms of type 1 diabetes prevention and therapy with the overall goal of improving β cell health during autoimmunity. OBJECTIVES/GOALS: Type 1 diabetes (T1D) results from immune-mediated destruction of pancreatic β cells. Recent data suggest that activation of senescence and acquisition of a senescence associated secretory phenotype (SASP) by β cells may contribute to T1D pathogenesis. However, the molecular mechanisms responsible for this phenotype are not well understood. METHODS/STUDY POPULATION: We hypothesize that loss of endoplasmic reticulum (ER) Ca²⁺ induces β cell senescence, SASP as well as mitochondrial dysfunction which drive T1D development. The current study utilizes SERCA2 KO INS-1 β cells (S2KO) exhibiting loss of ER Ca²⁺ and a SERCA2 haploinsufficient mice on a non-obese diabetic

background (NOD-S2+/-) to test the role of ER Ca²⁺ loss during T1D development. Senescence associated β galactosidase staining (SA- β gal), expression of senescence markers (RT-qPCR), mitochondrial function (Seahorse, TMRM) and mitochondrial copy number (qPCR) were all measured in S2KO versus WT β cells and are currently being measured in the NOD-S2+/- mouse model at 6, 8, 12, 14, and 16wks of age. RESULTS/ANTICIPATED RESULTS: RT-qPCR assays detecting senescence markers *cdkn1a* and *cdkn2a* and mitochondrial specific genes *cox1* and *nd1* were developed and validated in both INS-1 β cells and mouse islets. Mitochondrial function assay (Seahorse) was optimized for use in INS-1 β cells and is currently under development for use in intact mouse islets. S2KO β cells displayed increased SA- β gal staining as well as increased mitochondrial coupling efficiency ($p=0.0146$) and baseline mitochondrial copy number ($p=0.0053$) compared to WT β cells, suggesting a senescence phenotype and altered mitochondrial function. NOD-S2+/- mice exhibited increased expression of the senescence marker *cdkn2a* in the islet at 12wks ($p=0.0117$) compared to control mice, whereas *cdkn1a* remained unchanged across all timepoints tested. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our results suggest that loss of SERCA2 and reduced ER Ca²⁺ alter β cell mitochondrial function and are associated with features of senescence. Future studies will test whether SERCA2 activation and/or senolytic/senomorphing drugs are able to prevent or delay diabetes onset in NOD-S2+/- mice.

70759

Jaw-specific control of *Msx1*-dependent odontogenesis by *Dkk2* and *Sostdc1*

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ABSTRACT IMPACT: Our proposed jaw-specific control mechanism of tooth development is expected to address the site-specific prevalence of tooth agenesis in humans. OBJECTIVES/GOALS: To determine the molecular mechanisms that control jaw-specific tooth development. To identify the molecular basis of the site-specific prevalence of humans tooth agenesis cases. METHODS/STUDY POPULATION: We used three different genetically engineered mouse lines: ****Msx1*^{-/-}, *Dkk2*^{-/-}, and *Sostdc1*^{-/-} mice. We used developmental mouse genetics approaches, basically generating different combinations of compound mutant mice. We examined their tooth development by using gross, histology, and mRNA expression analyses. RESULTS/ANTICIPATED RESULTS: We identified that *Sostdc1*, a secreted Wnt inhibitor, also plays an important role in regulating the *Msx1*-dependent odontogenic pathway. *Sostdc1* mRNA showed similar expression patterns in the developing tooth germs between control and *Msx1*-null molar buds. Remarkably, by deleting the *Sostdc1* gene, as well as the *Dkk2* gene, in the *Msx1*-null background mouse, molar tooth development was rescued in the maxillary jaw, but not in the mandibular jaw. Furthermore, tooth developmental rescue could be achieved in both the maxillary and mandibular molars by combinedly deleting *Dkk2* and *Sostdc1* in *Msx1*-null mice. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our study demonstrates that secreted Wnt inhibitors *Dkk2* and *Sostdc1* synergistically regulate the *Msx1*-dependent odontogenic pathway and further control early tooth morphogenesis. These mouse model will be used to further address the site-specific prevalence of tooth agenesis in humans.

72399

Epigenetic Modification of Macrophages Contribute to Protective Memory in Against *Staphylococcus aureus*

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ABSTRACT IMPACT: This work may provide new targets for vaccine and immunotherapeutic development against MRSA infections. OBJECTIVES/GOALS: *Staphylococcus aureus* is the leading cause of skin and skin structure infection (SSSI), a primary portal of entry for invasive infection. Patients with SA SSSI have a high 1-year recurrence. We have shown innate memory protects mice against SA SSSI. The goal of this project is to determine epigenetic mechanisms of protective memory against SA SSSI. METHODS/STUDY POPULATION: We have shown macrophages (M ϕ) afford protective memory against recurrent SA SSSI in mice. Priming by prior infection reduced skin lesion size and MRSA burden, which correlated with increased M ϕ in abscesses and lymph nodes. Priming potentiated the opsonophagocytic killing of SA by bone-marrow derived M ϕ (BMDM) in vitro, and their adoptive transfer into naive skin afforded protective efficacy in vivo. Here, we investigated epigenetic mechanisms of anti-SA efficacy in BMDMs. BMDM from naive (uninfected) or primed (SA SSSI) wild-type C57Bl/6 mice were cultured ex vivo. DNA from BMDM groups were isolated and analyzed for methylation changes using reduced representation bisulfite sequencing (RRBS). Pathway analyses of methylation changes were determined with Panther. RESULTS/ANTICIPATED RESULTS: Present findings indicate the protective memory afforded by BMDM was mediated by epigenetic modifications of the DNA. Using RRBS, we profiled differentially methylated regions (DMR) in DNA from naive vs. primed BMDM. Primed BMDM exhibited significantly different DMRs as compared to naive BMDM. Proximity to known genes were mapped using GREAT. Pathway analyses revealed DMRs predominant in genes integral to immune modulation, such as integrin signaling, cytokine/chemokine networks, and growth regulation. For example, SA-primed BMDM were hypermethylated proximate to *GIMAP8* versus naive BMDM, suggesting repression of this protein. *Gimap* family ligands are small GTPase immune-associated proteins expressed in immune cells known to regulate macrophage lysosomal fusion during parasite infection. DISCUSSION/SIGNIFICANCE OF FINDINGS: These findings reveal epigenetic mechanisms of macrophage innate memory against recurrent MRSA infection. Functional testing of these genes in response to SA infection is needed to confirm their protective role. These insights may provide new targets for vaccine and immunotherapeutic development against MRSA.

79664

Complement Driven Auto-Reactive Antibodies in Lung Transplantation*

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ABSTRACT IMPACT: Our work unveils a novel mechanism of ischemia reperfusion injury driven by pre-existing autoimmunity following lung transplant and a potential therapeutic strategy for